

vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;

wherein the vector or vectors of (h)(i) and (h)(ii) are introduced into the incubating embryogenic callus by microprojectile-mediated delivery of the vector or vectors into the callus;

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- (i) culturing said transformed embryogenic callus containing embryos on selection medium; followed by
  - (j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent to yield a transformed embryo;
  - (k) culturing said transformed embryo on maturation medium; and
  - (l) recovering a transgenic plant from said transgenic embryo.

#### REMARKS

Claims 113-118 have been canceled as being redundant, not further limiting the claims from which they depend.

Claims 1, 6, 39, 80, 101, 102, and 103 have been amended to further clarify what is being claimed.

#### **Rejection of claims 1-45, 47, 72, 80, 102-103, 105-111, and 113-118 under 35 U.S.C. § 112, second paragraph**

In view of the foregoing amendments, the applicants respectfully submit that the § 112, second paragraph, rejections are obviated and respectfully request reconsideration and withdrawal of the rejections.

#### **Rejection of claims 6-37, 39-45, 47-71, 73-96, 98-100, 102-103, 105-106, 108-112, 114-115, and 117-118 under 35 U.S.C. § 112, first paragraph**

The claims were rejected as non-enabled for *Agrobacterium tumefaciens*-mediated transformation. The applicants respectfully traverse.

The burden is on the Patent Office to supply scientific evidence or reasoning as to why the claims are not enabled for their full scope:

As a matter of Patent Office practice, ... a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented **must** be taken as in compliance with the enabling requirement of the first paragraph of § 112 **unless** there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.

...  
[I]t is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain **why** it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement.

*In re Marzocchi*, 169 U.S.P.Q. 367, 369-370 (C.C.P.A. 1971). The applicants respectfully submit that the Office Action does not provide the requisite evidence or reasoning.

The Office relied upon Follansbee *et al.* for the allegation transformation of poinsettia is unpredictable, asserting that Follansbee *et al.* taught that whole *Euphorbia pulcherrima* (poinsettia) cannot be recovered following *A. rhizogenes* transformation. But *Agrobacterium rhizogenes* is not *Agrobacterium tumefaciens*. The difference is significant because *Agrobacterium rhizogenes* systems are designed for obtaining transgenic roots, not whole plants. *Agrobacterium tumefaciens* systems, on the other hand, have been developed to obtain whole plants, such as presently disclosed and claimed. Those skilled in the art would not consider Follansbee *et al.*'s work with *Agrobacterium rhizogenes* indicative or predictive of the likely outcome with *Agrobacterium tumefaciens* systems. Furthermore, the Office has supplied no evidence or scientifically valid reasoning why the results disclosed by Follansbee regarding *Agrobacterium rhizogenes* are relevant to the present claims, which recite transformation with a different bacterial species, *Agrobacterium tumefaciens*.

The Office further cites Oran and Ceasar in support of the assertion that *Agrobacterium tumefaciens*-mediated transformation is unpredictable and unlikely given the alleged host range limitations of the bacterium and the failure of any workers to report successful transformation of poinsettia via *Agrobacterium tumefaciens*. Oran teaches that an aqueous extract of *Euphorbia peplis* L. inhibited infection of potato tumors by *Agrobacterium tumefaciens*. But *Euphorbia peplis* L. is not *Euphorbia pulcherrima* (poinsettia) as recited in the present claims, and the Office has provided no evidence or

scientifically valid reasoning that this result with *Euphorbia peplis* L. has any relevance to the ability of *Agrobacterium tumefaciens* to facilitate transformation of *Euphorbia pulcherrima* (poinsettia).

Caesar was alleged to teach that there are few strains of *Agrobacterium tumefaciens* that successfully infect another *Euphorbia* species, and that no strains were previously reported to be infective. This is not accurate. What Caesar teaches, *inter alia*, is that among the 240 strains of *Agrobacterium tumefaciens* isolated from eastern North Dakota and Montana samples of the noxious weed species leafy spurge (*Euphorbia esula* L.), all 17 pathogenic strains from the Montana sample were pathogenic to leafy spurge and 3 of the 17 pathogenic strains isolated from the North Dakotan leafy spurge sample were pathogenic to leafy spurge. But *Euphorbia esula* L. is not *Euphorbia pulcherrima* (poinsettia) as recited in the present claims, and the Office has provided no evidence or scientifically valid reasoning that this result with *Euphorbia esula* L. has any relevance to the ability of *Agrobacterium tumefaciens* to facilitate transformation of *Euphorbia pulcherrima* (poinsettia).

Furthermore, the Office noted that Caesar stated that this was the first report of crown gall diseases (which results from *Agrobacterium tumefaciens* infection) of leafy spurge (and Russian Knapweed, the other plant studied). But, again, leafy spurge is not poinsettia, and poinsettia has long been known to be susceptible to crown gall disease (see attached.) And *Agrobacterium tumefaciens* is known to infect plants by transformation of DNA. Accordingly, one would expect *Agrobacterium tumefaciens* to be as successful a transformation vehicle for poinsettia as it has been for numerous other plant species.

In summary, the Office Action bases the instant rejection on teachings of one reference disclosing a different bacterium than recited in the present claims and two references disclosing results with different plants species than recited in the present claims. The Office Action fails to provide scientifically supported and acceptable evidence or reasoning why the cited references have any relevance to the present claims. The unstated implication appears to be that the bacterium studied by Follansbee and the plant species studied by Oran and Caesar are of the same genus as the bacterium and plants species (respectively) recited in the present claims and, therefore, one would expect the same results for all members of the genus. The applicants respectfully submit that there is no scientific basis for such an assumption.

Lastly, the Office asserts that (1) other methods of transformation, such as electroporation and polycation incubation of protoplasts, are dependent on whole plant regeneration from protoplasts or single cells and are not available to poinsettia and (2) tissue culture techniques developed for poinsettia have traditionally been genotype-dependent. The Office Action did not explain and the applicants fail to understand how these observations have any relevance as to whether one can obtain whole transformed poinsettia plants via *Agrobacterium tumefaciens* transformation.

In view of the foregoing, the applicants respectfully request reconsideration and withdrawal of the present rejection.

**Rejection of claims 73-75, 83, and 85 under 35 U.S.C. § 112, first paragraph**

Claims 73-75, 83, and 85 were rejected for lacking written description support. Specifically, the Office asserted that the claims are broadly drawn to any transgenic poinsettia plant containing any heterologous coding sequence conferring any trait but that only specific coding sequences conferring a limited set of traits was disclosed. The Office relied on *University of California v. Eli Lilly and Co.* for the propositions that (1) to satisfy the written description requirement, one must provide a precise definition of the claimed subject matter to distinguish it from other materials, (2) "naming a type of material generally known to exist, in the absence of knowledge as to what that material consists of, is not a description of that material," and (3) to claim a genus the patentee must provide a representative number of species of the claimed genus so that one skilled in the art could 'visualize or recognize the identity of the members of the genus." For the reasons below, the applicants traverse.

The applicants respectfully submit that the Office has misapplied the Court's holding in *Eli Lilly*. In *Eli Lilly*, the Federal Circuit considered whether a claim to a genus of nucleic acid sequences encoding a protein having a known function satisfied the written description requirement when only a small handful of sequences within the genus were disclosed. The court held that in such instances defining physical characteristics of the claimed sequences were required; merely reciting the function of the sequence was insufficient. In *Enzo Biochem, Inc., v. Gen-Probe, Inc.*, 296 F.3d 1316, 1324, 63 U.S.P.Q.2d 1609, 1613 (Fed. Cir. 2002), the Federal Circuit clarified its *Eli Lilly* holding by stating

that reciting a function may be sufficient to meet the written description requirements if there is a known correlation of function to a particular, known structure.

More recently, the Federal Circuit considered whether claims to EPO produced by vertebrate (particularly mammalian) cells and processes for producing the EPO from those cells satisfied the written description requirement. *Amgen Inc. v. Hoechst Marion Roussel, Inc.*, 2003 U.S. App. LEXIS 118 (Fed. Cir. Jan. 6, 2003). The defendant argued that the plaintiff, Amgen, failed to provide written description support for the terms "vertebrate cells" and "mammalian cells." In rejecting the defendants' challenge, the Court stated:

Both *Eli Lilly* and *Enzo Biochem* are inapposite to this case because the claim terms at issue here are not new or unknown biological materials that ordinarily skilled artisans would easily miscomprehend. Instead, the claims of Amgen's patents refer to types of cells that can be used to produce recombinant human EPO. Thus, TKT [a defendant] can only challenge the adequacy of disclosure of the vertebrate or mammalian host cell – not the human DNA itself. This difference alone sufficiently distinguishes *Eli Lilly*, because when used, as here, merely to identify types of cells (instead of undescribed, previously unknown DNA sequences), the words "vertebrate" and "mammalian" readily "convey[] distinguishing information concerning [their] identity" such that one of ordinary skill in the art could "visualize or recognize the identity of the members of the genus." *Eli Lilly*, 119 F.3d at 1567, 1568, 43 USPQ2d at 1406.

*Amgen*, 2003 U.S. App. LEXIS 118, at \*41-42.

As in *Amgen*, the terms objected to in the presently rejected claims do not describe previously unknown things. It is not incumbent on the applicants to describe all the foreign genes (a multitude of which the specification makes clear were known in the art, see pages 28-42 of the specification, which are dedicated to disclosing a representative example of foreign genes). Unknown foreign genes are not the applicants' invention. Rather, the claims are drawn to transformed poinsettia plants, and the specification must supply adequate written description of the plants such that one skilled in the art could envision the claimed plants and understand that the applicants had possession of them. Just as the Federal Circuit held in *Amgen* that the terms "mammalian cells" and "vertebrate cells" are well known and therefore not new or unknown biological materials that the ordinary skilled artisan would easily misapprehend, "foreign genes" does not describe new or unknown biological materials that the ordinary skilled artisan would easily misapprehend. The applicants respectfully submit that based on the present specification, one of ordinary skill in the art would have no difficulty

(a) envisioning a poinsettia plant transformed with any of a multitude of foreign genes (numerous ones being disclosed in the specification), and (b) understanding that the applicants, who for the first time demonstrated success in obtaining whole transformed poinsettia plants, contemplated and had possession of all such transformed plants.

The applicants' invention is in essence the combination of two known things (poinsettia plants and genes foreign to the poinsettia plants) that until now could not be combined. And it is well settled that an applicant need not describe that which is well known in the art. *E.g., Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 94 (Fed. Cir. 1986) ("a patent need not teach, and preferably omits, what is well known in the art.")

In view of the foregoing, the applicants respectfully request reconsideration and withdrawal of the pending rejection.

#### **Rejection of claims 1, 97, 101, 104, 113, and 116 under 35 U.S.C. § 103**

Claims 1, 97, 101, 104, 113, and 116 were rejected under 35 U.S.C. § 103(a) as being obvious over Preil taken with Lelu *et al.* and DeWald *et al.* in light of Hartmann *et al.* and Lee. For the following reasons, the applicants respectfully traverse this rejection.

1. *The cited art fails to teach or suggest all the elements recited in the pending claims*

The prior art fails to teach or suggest the claimed multi-step processes for poinsettia regeneration. For example, claim 1 recites a method comprising:

- (a) incubating a poinsettia plant tissue explant that produces a reddish epidermal callus on auxin- and cytokinin-containing callus induction medium;
- (b) subculturing the reddish epidermal callus to embryo induction medium comprising a nitrogen source to form an embryogenic callus;
- (c) culturing said embryogenic callus on developmental medium containing an osmotic pressure increasing agent and a cytokinin; followed by
- (d) culturing said embryogenic callus on maturation medium to yield an embryo; and
- (f) recovering a poinsettia plant from said embryo.

By contrast, the primary reference relied upon for this rejection, Priel et al., teaches at p. 50, 1<sup>st</sup> column, placing stem segments on "callus induction medium" followed by transfer to "somatic embryogenesis induction medium" where, after transfer to fresh medium, the first embryogenic structures become visible. The embryogenic structures are then placed on "somatic embryo maturation medium," where they reach the cotyledonary stage.

But neither Priel nor the other cited art teach or suggest placing the poinsettia embryogenic callus on both a developmental medium and a maturation medium; Priel teaches only placing the embryogenic structures on a single medium.

Thus, even were one to combine the teachings of the prior art, one would not arrive at the instantly claimed methods. And there is not even a specific allegation in the Office Action that using both a developmental medium and a maturation medium or casein hydrolysate in the embryo induction medium for somatic embryogenesis would have been obvious to one of ordinary skill in the art.

2. The prior art fails to teach or suggest the particular combination of elements now being claimed

The applicants respectfully submit that the Examiner has relied on particular teachings that were selectively culled from the prior art without any suggestion in the references themselves or reason why such selections would have distinguished themselves to those of ordinary skill in the art. Obviousness cannot be predicated on the mere identification of elements in the prior art – a clear identification as to the reason one of ordinary skill in the art would have selected these elements must be made. *In re Kotzab*, 55 USPQ2d 1313 (Fed. Cir. 2000).

There is simply no teaching or suggestion to make the various combination of modifications to prior art methods of *in vitro* regeneration of poinsettia as presently claimed. One cannot establish a *prima facie* case of obviousness without identifying in the art a suggestion or motivation to make the particular invention being claimed. *In re Deuel*, 51 F.3d 1552, 1559 (Fed. Cir. 1995) ("A general incentive does not make obvious a particular result, nor does the existence of techniques by which those efforts can be carried out."); and *Ex parte Obukowicz*, 27 U.S.P.Q.2d, 1063, 1065 (Bd. Pat. App. Int. 1992) (Prior art "that gives only general guidance and is not at all specific as to the

particular form of the claimed invention and how to achieve it . . . does not make the invention obvious.”).

3. The cited art fails to imbue the ordinary artisan with a reasonable expectation of success

In the July 7, 1999, Office Action, the Examiner noted, “[Obtaining] whole poinsettia plants from tissue culture is unpredictable, given the highly genotype-dependent techniques available at the time of the invention and the recalcitrance of transformed *Euphorbia* cells to produce whole plants.” Given this unpredictability, there could not have been a reasonable expectation of successfully making and using the presently claimed method.

Furthermore, the prior art includes examples of failures of others. In the September 25, 1998, Office Action, the Examiner applied Cheetham against the claims in an obviousness rejection. Cheetham attempted to regenerate shoots from cultured root explants of poinsettia, but they never succeeded (the publication expressly stating on p. 513 that “no shooting was ever observed.” Furthermore, the applicants submitted correspondence between one of the inventors and Dr. P. Weathers, one of the authors of the Cheetham article in which Dr. Weathers states, “We tried various regimens of hormones to get plant regeneration . . . . No shoots were ever observed.” The applicants subsequently submitted the biography of Dr. Weathers, which identified her as being of at least ordinary skill in the art. Given the failure of others, one of ordinary skill in the art could not have had a reasonable expectation of success.

Without a reasonable expectation of success, the claim invention cannot be obvious. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991).

4. The cited art fails to teach or suggest the inherent advantages of the claimed method

There is nothing in the prior art that suggests to the ordinary artisan the other inherent advantages of the presently claimed methods. For example, page 15 of the present specification states that the presently claimed methods are genotype-independent vis-à-vis producing large quantities of somatic embryos of improved quality and yield. Such inherent properties of the present methods were not recognized in the art.



Furthermore, the present specification notes at page 16, lines 7-9, that "[t]his additional treatment [i.e., the combination of culturing the embryogenic callus on a developmental medium and maturation medium] improves embryo germination uniformity and confers a high degree of desiccation tolerance." This inherent advantage in the presently claimed methods is also neither taught nor suggested by the cited art.

For all of the foregoing reasons, the applicants respectfully request that this § 103 rejection be withdrawn.

Claims 1, 97, 101, 104, 113, and 116 were rejected under 35 U.S.C. § 103(a) as being obvious over Preil taken with Lelu *et al.* and DeWald *et al.* in light of Hartmann *et al.* and Lee and further in view of Nataraja and Litz. For the following reasons, the applicants respectfully traverse this rejection.

This rejection is substantively the same as the previous rejection, with the addition of Nataraja and Litz applied for their teachings regarding casein hydrolysate. But Nataraja and Litz do not compensate for the shortcomings of Preil taken with Lelu *et al.* and DeWald *et al.* in light of Hartmann *et al.* and Lee detailed above. That is, this combination of references:

1. does not yield the claimed invention as none of the references, alone or in combination teach or suggest placing the poinsettia embryogenic callus on both a developmental medium and a maturation medium;
2. does not teach or suggest the particular multi-step processes claimed or the particular compositions used in each step;
3. does not provide a reasonable expectation of success; and
4. does not teach or suggest the inherent advantages of the claimed method.

Accordingly, the applicants respectfully request reconsideration and withdrawal of this rejection.

**Rejection of claims 1-3, 6-8, 11-41, 44-45, 47-106, and 108-118 under 35 U.S.C. § 103(a)**

Claims 1-3, 6-8, 11-41, 44-45, 47-106, and 108-118 were rejected as obvious over Miki *et al.* with Preil *et al.* taken with Lelu *et al.* and Dewald *et al.* in light of Harmann *et al.* and Lee *et al.*, further in view of Nataraja and Litz. This rejection is substantively the same as the previously discussed


rejections with the additional reliance on Miki *et al.* for its teaching of particle bombardment. Yet Miki *et al.* does not cure the deficiencies of the remaining references as detailed above. That is, the combination of Miki *et al.* with Preil *et al.* taken with Lelu *et al.* and Dewald *et al.* in light of Harmann *et al.* and Lee *et al.*, further in view of Nataraja and Litz:

1. does not yield the claimed invention as none of the references, alone or in combination teach or suggest placing the poinsettia embryogenic callus on both a developmental medium and a maturation medium;
2. does not teach or suggest the particular multi-step processes claimed or the particular compositions used in each step;
3. does not provide a reasonable expectation of success;
4. does not teach or suggest the inherent advantages of the claimed method.

In view of the foregoing, the applicants respectfully request reconsideration and withdrawal of this § 103 obviousness rejection.

If there are any questions or comments regarding this Response or application, the Examiner is encouraged to contact the undersigned attorney as indicated below.

Respectfully submitted,



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**APPLICATION SERIAL NO. 08/903,944**

**Redlined Version of Amended Claims**

1. (Three Times Amended) A method for in vitro regeneration of poinsettia plants comprising:
  - (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus on auxin- and cytokinin-containing callus induction medium;
  - (b) subculturing reddish epidermal callus to embryo induction medium comprising casein hydrolysate and further comprising  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  to form embryogenic callus;
  - (c) culturing said embryogenic callus on developmental medium containing an osmotic pressure increasing agent and cytokinin;
  - (d) culturing said embryogenic callus on maturation medium comprising abscisic acid; and
  - (e) recovering poinsettia plants from said embryos.
  
6. (Four Times Amended) A method for producing transgenic poinsettia plants, comprising:
  - (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus on auxin- and cytokinin-containing callus induction medium;
  - (b) culturing reddish epidermal callus on embryo induction medium comprising casein hydrolysate and further comprising  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  to form embryogenic callus;
  - (c)
    - (i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
    - (ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;wherein the vector or vectors of (c)(i) and (c)(ii) are introduced into the incubating embryogenic callus by co-incubating the callus with *Agrobacterium tumefaciens* containing the vector or vectors or by microprojectile-mediated delivery of the vector or vectors into the callus;
  - (d) culturing said transformed embryogenic callus on selection medium;

- (e) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
- (f) culturing said transgenic embryos on maturation medium; and
- (g) recovering transgenic plants from said transgenic embryos.

39. (Five Times Amended) A method for producing transgenic poinsettia plants, comprising:

- (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus in auxin- and cytokinin-containing callus induction medium;
- (b) subculturing embryogenic callus produced on said callus induction medium to liquid  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  containing embryo induction medium comprising casein hydrolysate;
- (c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;
- (d) filtering the culture and culturing the filtrate on solid embryo induction medium;
- (e) subculturing embryos produced on said embryo induction medium to maturation medium;
- (f) culturing said embryos on callus induction medium;
- (g) subculturing epidermal callus produced on said callus induction medium to embryo induction medium to form embryogenic callus;
- (h)
  - (i) introducing an expression vector into said embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
  - (ii) introducing two expression vectors into said embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;

wherein the vector or vectors of (h)(i) and (h)(ii) are introduced into the incubating embryogenic callus ~~by co-incubating the callus with *Agrobacterium tumefaciens* containing the vector or vectors or by microprojectile-mediated delivery of the vector or vectors~~ into the callus;

- (i) culturing said transformed embryogenic callus on selection medium;

- (j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
  - (k) culturing said transformed embryos on maturation medium; and
  - (l) recovering transgenic plants from said transgenic embryos.
80. (Amended) The transgenic poinsettia plant of claim 76, wherein said foreign gene confers resistance to a bacterium or a fungus, and ~~wherein said second foreign gene encodes a~~ polypeptide selected from the group consisting of chitinase, a  $\beta$ -1,3-glucanase, ribosome-inactivating protein, lytic peptide, and plant defensin.
102. (Three Times Amended) A method for producing transgenic poinsettia plants comprising the steps of:
- (a) incubating poinsettia plant tissue explants that produce epidermal callus on auxin- and cytokinin-containing callus induction medium;
  - (b) subculturing embryogenic callus to embryo induction medium comprising casein hydrolysate and further comprising  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  to form embryogenic callus containing embryos;
  - (c)
    - (i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
    - (ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;wherein the vector or vectors of (c)(i) and (c)(ii) are introduced into the incubating embryogenic callus by co-incubating the callus with *Agrobacterium tumefaciens* containing the vector or vectors or by microprojectile-mediated delivery of the vector or vectors into the callus;
  - (d) culturing said transformed embryogenic callus on selection medium;
  - (e) culturing said embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;

- (f) culturing said transgenic embryos on maturation medium; and
- (g) recovering transgenic plants from said transgenic embryos.

103. (Twice Amended) A method for producing transgenic poinsettia plants comprising the steps of:

- (a) incubating poinsettia plant tissue explants that produce epidermal callus on auxin- and cytokinin-containing callus induction medium;
- (b) subculturing embryogenic callus produced on said callus induction medium to liquid embryo induction medium comprising casein hydrolysate and further comprising  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$ ;
- (c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;
- (d) filtering the culture and culturing the filtrate on solid embryo induction medium;
- (e) subculturing embryos produced on said embryo induction medium to maturation medium;
- (f) culturing said embryos on callus induction medium;
- (g) subculturing embryogenic callus produced on said callus induction medium to embryo induction medium to form embryogenic callus containing embryos;
- (h)
  - (i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
  - (ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;
 wherein the vector or vectors of (h)(i) and (h)(ii) are introduced into the incubating embryogenic callus by microprojectile-mediated delivery of the vector or vectors into the callus;
- (i) culturing said transformed embryogenic callus on selection medium;
- (j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;

- (k) culturing said transformed embryos on maturation medium; and
- (l) recovering transgenic plants from said transgenic embryos.